IMPORTANCE OF RURAL PRODUCTION TECHNOLOGY OF BGA (*AULOSIRA, TOLYPOTHRIX, ANABAENA, NOSTOC)*

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1. ABSTRACT

Almost BGA are found in every type of habitat and so that they are a morphologically diversely and large group of phototrophic prokaryotes on the earth. The remarkable lack of morphological change may explain by this versatility has been seen in fossilized cyanobacteria which are 3.5 billion years old and in their counterparts of modern days. An important part of algae will make excellent fertilser for paddy, and will have food supplement for livestock with high protein especially for the poultry in India. This coupled will definitely encourage production of algae- feed-fertilizer integration raising in the recycling system of nitrogen with livestock. Uses of BGA as a fertilizer provides inherent advantage ecologically with cyclic nutrient supply system. For the development of biofertiliser technology based on BGA in India, a considerable progress has been made. This point also has been raises in demonstration that this technology may be a important and powerful source of increasing rice crop yield with enriching fertility of soil. But, however this technology also needs to be further improvement system for exploitation in better way under the sustainable system of agriculture. It is also necessary to find out so much detail understanding in agriculture system, dynamics of algal population over the whole annual cycle. Field studies at extensive level to developing high quality inoculums at region specific are also required. BGA may be very useful to understand biology of drought resistant in the term of extending this type of approach to dry crops.

Keywords: BGA, diversely, phototrophic prokaryotes, fertilizer, poultry, biofertiliser technology, agriculture system, dry crops etc.

2. INTRODUCTION

In the waterlogged conditions of agricultural land the BGA find a highly favorable adobe and also BGA very cheap nitrogen besides increasing crop yield to plants by making soil vital, productive and fertile. BGA helps in creating a safe agro-ecosystem environmentally that also ensures economic viability in rice cultivation which are known as 'Algalization'- a BGA biofertlilizer and it is also make effect in other various crops while saving energy intensive inputs. Rice is used the most staple food crop for more than one third population of the world and it is also the oldest cultivated crops of the world. To increase the rice productivity the inoculation of nitrogen fixing BGA are a sustainable and also alternative source of nitrogen. The mixed inoculums of Anabaena, Aulosira, Tolyopothrix and Nostoc were used in the field. For rice productivity the Blue Green Algae (BGA) inoculums was found to be most effective treatment as reported by *Gurung, (2004); Paudel, et.al.,(2012)*. BGA are important in helping to maintaining fertility of paddy fields through nitrogen fixation and while present abundantly in paddy

fields and involved in the photosynthesis. Ultrastructure of cyanobacterial cell wall, biochemical and pigment analysis of blue green algae were isolated from the paddy *(Thamizh & Sivakumar, 2012)*. *Watanabe,et.al.(1981); Grant,et.al.,(1983)* research shows deliberate manipulation possibility of the ecosystem to favour BGA by limiting. Surface application of straw, phosphorus application and grazer control. The ability of some BGA forms to carry out nitrogen fixation and photosynthesis both confers on them an agricultural and ecological advantage as a natural resource of biological nitrogen. *Kulik, (1995)* showed the beneficial effects of bio-fertilizers are addition of nitrogen, increased soil organic matter and soil aggregation. The role of bio-fertilizers in sustainable agriculture recorded special significance, particularly in the present context of high cost of chemical fertilizers has been made by *Kannaiyan (2002)*. The production and application of bio-fertilizers to leguminous plants, oilseeds, rice, millets and forest nursery plants are very common in India as reported by many earlier workers *(Kannaiyan, 2002; Rai, et.al., 2004)*.

3. MATERIAL AND METHODS

Methods of increasing blue-green algal biomass

Inocula has grown in shallow trays and before that derived from a mixture of strains originally isolated from paddy fields and phosphate. To adjust pH of soil at 7.0-7.5 some lime is added, if required. The BGA mats developed and has make completely dried as well as a well established fact. There are so many studies have been reported as increased grain yield. Straw nitrogen content or grain nitrogen content with the role and effect of BGA being equivalent addition of 20 to 30 kg ha-u nitrogen provided and phosphorus fertilizer is also added *(Singh and Singh, 1987)*. From the paddy field about 5 gm soil from the top is taken and then hundred ml of fogg's medium added in a flaks and after that shaken it well , and incubated at the temperature of room. Now, to increase the BGA growth 1500 lux illumination is provided to the culture. After that with the help of a loop, algal culture is transferred to ten ml of water in a tube but tube should be shaken well so that algal filaments can be separated completely. The content diluted seriously, and to establish algal growth, drop of each dilution is inoculated into Fogg's medium. This whole process executed in a Petridis. .**Fogg's medium-**

KH_2PO_2 - 0.2 g;	$MgSO_4.7H_20$ - 0.2 g;	$CaCl_2$ - 0.1 g;
Na_2MoO_4 - 0.1 mg;	$MgCl_2$ - 0.1 mg;	H_3BO_3 -0.1 mg;
$CuSO_4$ - 0.1 mg;	$ZnSO_4$ - 0.1 mg;	Fe-EDTA - 1.0 ml;
	Distilled water - 100 ml; pH - 7	

From the each culture a drop is examined in microscope. It is used further only when it is supposed to be pure culture for a single species. The sample is diluted till the time of isolation of pure culture is attained in case of more than one species. For the growth of BGA pure culture are transferred to culture flask having with Fogg's medium and enough light is provided for the support of growth. Now, algal culture is used as starter culture and after that this is also initiates BGA mass culture. Processed can be in four ways – Iron and Zink are used in library as trough. These are 22 cm height and 2×3 size. The trough is filled with 200gm super phosphate 10-12 kg of soil is spread in soil and after that water is poured upto 5-15 cm of height. To adjust the pH about 7 calcium carbonate is added and provided saw dust to soil. Now, sprinkle over it starter culture. BGA is developed very nicely after trough is kept in sunlight and it is watered each day. After a good growth the soil is got to dry and then dry flakes are collected and packed safely for algalization.

Pit Method

Shallow pits are maintain under the full sunlight. Polythene sheets are lined inside the pit to avoid the perlocation. The pit is watered with 10 cm in height after filling of 20 cms soil in pit and carbofuran is added to pit after maintaining of pH. The soil is sprinkled with the starter culture after spreading saw dust over the soil. To favour the growth of BGA pits are watered and after that the soil is allowed to get dry.

Field Method

A 40 square meter of small plot in open field are maintained. 20 Kg superphosphate is added in field after plot is watered upto 15 cms. 240 gm carbofuran is added in field after correcting the pH after that the starter culture about 5 kg is provided to frequently watered plot. During three to four weeks BGA developed and after that soil is allowed to get dry. About thirty kg BGA inoculants can be harvested through this method.

Measurements of Nitrogen Fixation

Using the acetylene reducing activity (ARA) method, N2 fixation by BGA has been most frequently studied which may provide erroneous results. During the day ARA Variations the growing cycle can be rapid, important and ARA has a log-normal distribution *(Roger, et al., 1977)*. Therefore, very frequent measurement and many replicates are required to measure total ARA satisfactorily. Because of the conversion factor of acetylene

nitrogen is not constant this tedious work will lead to an imprecise evaluation of the nitrogen activity (NFA) and needs to be determined *(Peterson and Burris, 1976)*. But when the measurement is brief ARA is a very reliable and convenient method foe qualitative study *(David and Fay, 1977)*, when statistically valid methods are adopted and the problems of green house effects and gas diffusion are minimized *(Roger and Kulasooriya, 1980)*. The importance of anaerobic nonheterocytous Nitrogen fixing Cyanobacteria was not appropriate until recently. Under an aerobic gas phase the field measurement of nitrogenase activity were only carried out, therefore, to evaluate the Nitrogen inputs is difficult due to nitrogen fixation by nonheterocytous BGA *(Stewart, 1978)*. Data reported to BGA on BNF varied from a few to 80 kg N/ha and the averaged per crop 27 kg/ha *(Roger and Kulasooriya, 1980)*.

The Algal nitrogen fixing capacity

To show nitrogen fixation any experiment designed by a given organisms must be carried out in mind with points mentioned below:

For the fixing nitrogen the organism must be in a pure culture because, even if a contaminant is present which known to be otherwise it will be incapable to fixation of nitrogen, the possibility could not be precluded that it may fix nitrogen during the presence of the organisms which is being examined.

The species of *Anabaena, Aulosira, Nostoc & Tolypothrix* are generally used for starter inoculums. For the mass cultivation The below mentioned four methods are used *(a)* Tank method (cemented), *(b)* shallow metal troughs method, *(c)* polythene lined pit method, and *(d)* field method. For the small and for the farmers of marginalized group the most suitable method is the polythene–lined pit method to prepare algal biofertilizer. In the polythene line pit method a small pit are prepared and after that lined with polythene thick sheets. Mass production of BGA can be done to use of any of method mentioned below. The steps are as follows:

(1) In an open area prepare a shallow tray of iron sheets, cemented tank or polythene lined pit. Pits or tank should be below to 1.5 m. in width. It would be facilitate handling of culture properly

(2) About 2-3 kg soil collected from the open place for l m^2 are of the tank and 100g of superphosphate added. In the pit about ten cm water level increased and to adjust the pH 7 add the lime. Add 2 ml of insecticide *e.g.* To protect the culture from mosquitoes malathion is required and to allow the settle down soils particles mix it very well.

(3) When water becomes clear, on the surface of water sprinkle 100 g of starter inoculums.

(4) When temperature remains between 35-40° maximum growth of cyanobacteria is achieved during the summer but water level should be maintained always about 10 cm during this particular time.

(5) The algal mat will get separate from the forms flakes and soil after drying. During the period of sunny days (Summer) near about one kg pure BGA mat per m^2 area is produced and these should be collected to kept before polythene bags get it powdered form to supply for the farmers

(6) If the same process is repeated the algal flakes can also be used as starter inoculums.

Pot culture Method

About 20 days the seeds of rice were soaked in water after that with the 2 cm of heights 5 seedlings were transferred to pots. 1 g mixed algal inoculums was added in the soil of pot one week before transferring of seedlings and then after one week of transferring seedlings. The height of plant, roots length fresh and dry weight were measured after three weeks as per method suggested by *Meloni, et.al.,(2004)*. Particle density, bulk density porosity of soil *(Blake and Hartage 1986)* and Moisture *(Hayes 1981)*, were also noted.

Temperature (°C)

Water temperature, were recorded with the help of maximum and minimum temperature on thermometer. These simple mercury thermometers are graduated from 0 degree to 50 degree Celsius and each degree with ten divisions.

4. RESULT & DISCUSSION

Biomass was expressed and dry weight (mg/ml) basis which showed that growth of all organism followed and increasing trend with increase in time of incubation. Maximum dry biomass was observed in Isolate-3 i.e. 0.85±0.0213 mg/ml, followed by *Tolypothrix tenius* 0.82±0.0197 mg/ml and *Nostoc muscorum* 0.79±0.0174 mg/ml whereas minimum dry biomass was observed in *Aulosira fertilissima* i.e. 0.04±0.0005 mg/ml, followed by *Anabaena variabilis*, 0.08±0.0012 mg/ml and Isolate-2, Isolate-4, 0.10 mg/ml.

Dry Biomass Estimation at 7 days incubation period, shown maximum in Isolate 3 i.e. 0.21±0.0053 mg/ml followed by *Tolypothrix tenius*, 0.17±0.0041 mg/ml whereas minimum value recorded 0.04±0.0005 mg/ml in *Aulosira fertilissima*, followed by *Anabaena variabilis* 0.08±0.0012 mg/ml and dry biomass estimation at 14 days incubation period, shown maximum in *Tolypothrix tenius* i.e. 0.32±0.0080 mg/ml followed by Isolate 3,

0.30±0.0072 mg/ml whereas minimum value recorded 0.13±0.0016 mg/ml in Isolate 4, followed by *Anabaena variabilis* 0.14±0.0021 mg/ml whereas and dry biomass estimation at 21 days incubation period, shown maximum in Isolate 3 i.e. 0.85±0.0213 mg/ml followed by *Tolypothrix tenius*, 0.82±0.0197 mg/ml whereas minimum value recorded 0.26±0.0031 mg/ml in Isolate 2, followed by Isolate 4, 0.32±0.0048 mg/ml. The result are tabulated in *Table No. 4.1*

Estimated total nitrogen in different cynobacterial cultures showed that the total nitrogen content (%) (TLC) increased with in time. Maximum total nitrogen (%) estimation was observed in *Anabaena variabilis* i.e. 0.0113±0.00028 mg/ml, followed by *Aulosira fertilissima* & *Tolypothrix tenius* i.e. 0.0075±0.00018 mg/ml whereas minimum total nitrogen (%) estimation was observed in Isolate 2 i.e. 0.0010±0.000012 mg/ml, followed by Isolate 3, *0.0012*±0.00001 mg/ml.

Table– 4.1

Dry Biomass Estimation (mg/ml) at different intervals of time

S. No.	Dry Biomass Estimation (mg/ml) at different intervals of time			
	Cyanobacterial strains	Mean ±SE (7 days incubation period)	Mean ±SE (14 days incubation period)	Mean ±SE (21 days incubation period)
01	*Anabaena variabilis*	0.08±0.0012	0.14±0.0021	0.37±0.0067
02	*Aulosira fertilissima*	0.04±0.0005	0.16±0.0029	0.63±0.0126
03	*Nostoc muscorum*	0.13±0.0029	0.23±0.0051	0.79±0.0174
04	*Tolypothrix tenius*	0.17±0.0041	0.32±0.0080	0.82±0.0197
05	Isolate 2	0.10±0.0018	0.19±0.0038	0.26±0.0031
06	Isolate 3	0.21±0.0053	0.30±0.0072	0.85±0.0213
07	Isolate 4	0.10±0.0020	0.13±0.0016	0.32±0.0048

Table–4.2

Total nitrogen (%) estimation of heterocystous cyanobacterial strains at different intervals of time

S. No.	Total nitrogen (%) at different intervals of time			
	Cyanobacterial strains	Mean ±SE (7 days incubation period)	Mean ±SE (14 days incubation period)	Mean ±SE (21 days incubation period)
01	*Anabaena variabilis*	0.0046±0.000115	0.0073±0.00018	0.0113±0.00028
02	*Aulosira fertilissima*	0.0017±0.000031	0.0070±0.00015	0.0075±0.00018
03	*Nostoc muscorum*	0.0013±0.000020	0.0017±0.00002	0.0055±0.00012
04	*Tolypothrix tenius*	0.0023±0.000046	0.0075±0.00019	0.0051±0.00010
05	Isolate 2	0.0010±0.000012	0.0028±0.00005	0.0038±0.00007
06	Isolate 3	0.0028±0.000067	0.0036±0.00007	0.0012±0.00001
07	Isolate 4	0.0023±0.000051	0.0018±0.00003	0.0029±0.00004

Table-4.3 Heterocyst frequency (%) of different cyanobacterial strains

Unit : %

S.	Cyanobacterial	Heterocyst frequency (%)

No	strains	S1	S2	S3	S4	S5	Mean ±SE
01	*Anabaena variabilis*	18.10 ±0.434	13.12 ±0.197	17.40 ±0.418	12.25 ±0.172	22.09 ±0.619	**16.59** ±0.368
02	*Aulosira fertilissima*	13.18 ±0.211	16.12 ±0.306	11.30 ±0.147	15.14 ±0.273	19.22 ±0.500	**14.99** ±0.287
03	*Nostoc muscorum*	15.21 ±0.289	13.30 ±0.213	14.19 ±0.241	21.27 ±0.574	18.24 ±0.456	**16.44** ±0.355
04	*Tolypothrix tenius*	22.10 ±0.619	21.09 ±0.548	27.40 ±0.767	30.10 ±0.873	28.25 ±0.819	**25.79** ±0.725
05	Isolate 5	10.25 ±0.113	14.27 ±0.257	18.24 ±0.456	16.30 ±0.342	21.23 ±0.573	**16.06** ±0.348
06	Isolate 6	16.20 ±0.324	13.10 ±0.197	19.40 ±0.504	11.28 ±0.147	15.20 ±0.289	**15.04** ±0.292
07	Isolate 7	12.21 ±0.171	17.23 ±0.362	14.10 ±0.226	21.20 ±0.572	19.09 ±0.477	**16.77** ±0.362
08	Isolate 8	17.24 ±0.379	13.05 ±0.183	10.28 ±0.123	14.40 ±0.259	18.20 ±0.455	**14.63** ±0.280
09	Isolate 9	11.27 ±0.147	10.40 ±0.125	13.50 ±0.216	09.95 ±0.109	14.25 ±0.257	**11.87** ±0.171
10	Isolate 10	13.10 ±0.197	11.24 ±0.146	17.27 ±0.397	12.28 ±0.172	14.10 ±0.240	**13.60** ±0.230

* S1, S2, S3, S4, & S5 are five different spots in the site.

Table-4.4 Dry Biomass Estimation (mg/ml) of Heterocystous cyanobacterial strains after 21 days of incubation

Unit : mg/ml

S. No.	Cyanobacterial strains	Dry biomass estimation (mg/ml)					
		S1	S2	S3	S4	S5	Mean ±SE
01	*Anabaena variabilis*	0.38 ±0.0057	0.15 ±0.0017	0.42 ±0.0063	0.46 ±0.0083	0.48 ±0.0091	0.38 ±0.0053
02	*Aulosira fertilissima*	0.45 ±0.0086	0.37 ±0.0052	0.43 ±0.0073	0.50 ±0.0100	0.65 ±0.0163	0.48 ±0.0101
03	*Nostoc muscorum*	0.43 ±0.0077	0.65 ±0.0169	0.54 ±0.0124	0.58 ±0.0145	0.63 ±0.0158	0.57 ±0.0131
04	*Tolypothrix tenius*	0.71 ±0.0192	0.69 ±0.0173	0.58 ±0.0139	0.75 ±0.0203	0.77 ±0.0216	0.70 ±0.0182
05	Isolate 2	0.94 ±0.0273	0.85 ±0.0247	0.92 ±0.0276	0.74 ±0.0192	0.63 ±0.0164	0.82 ±0.0230
06	Isolate 3	0.48 ±0.0096	0.56 ±0.0123	0.52 ±0.0109	0.62 ±0.0149	0.43 ±0.0069	0.52 ±0.0114
07	Isolate 4	0.30 ±0.0033	0.34 ±0.0044	0.29 ±0.0035	0.32 ±0.0038	0.31 ±0.0040	0.31 ±0.0037

* S1, S2, S3, S4, & S5 are five different spots in the site.

Table-4.5 Chlorophyll estimation (µg/ml) of heterocystous cyanobacterial strains after 21 days of incubation

Unit : µg/ml

S. No.	Cyanobacterial strains	Chlorophyll estimation (µg/ml)					
		S1	S2	S3	S4	S5	Mean ±SE
01	*Anabaena variabilis*	3.40 ±0.048	3.60 ±0.054	3.10 ±0.043	3.70 ±0.056	3.80 ±0.061	3.52 ±0.052
02	*Aulosira fertilissima*	5.10 ±0.117	5.70 ±0.137	4.90 ±0.108	5.27 ±0.126	5.12 ±0.118	5.22 ±0.121
03	*Nostoc muscorum*	6.14 ±0.154	6.90 ±0.186	6.60 ±0.172	6.13 ±0.153	6.10 ±0.146	6.37 ±0.162
04	*Tolypothrix tenius*	2.12 ±0.023	3.10 ±0.040	2.14 ±0.026	2.80 ±0.036	2.10 ±0.023	2.45 ±0.030
05	Isolate 2	7.13 ±0.193	7.60 ±0.213	7.14 ±0.200	6.48 ±0.168	7.24 ±0.203	7.12 ±0.195
06	Isolate 3	4.23 ±0.085	4.10 ±0.066	4.17 ±0.071	4.11 ±0.066	4.28 ±0.090	4.18 ±0.075
07	Isolate 4	4.30 ±0.095	4.12 ±0.070	4.25 ±0.085	4.28 ±0.090	4.90 ±0.113	4.37 ±0.090

* S1, S2, S3, S4, & S5 are five different spots in the site.

Table-4.6 Total Nitrogen Estimation(%) of *Heterocystous cyanobacterial* strains after 21 days of incubation

Unit : %

S. No.	Cyanobacterial strains	Total Nitrogen Estimation(%)					
		S1	S2	S3	S4	S5	Mean ±SE
01	*Anabaena variabilis*	0.0063 ±0.00014	0.0061 ±0.00013	0.0095 ±0.00027	0.0086 ±0.00022	0.0072 ±0.00017	0.0075 ±0.00019
02	*Aulosira fertilissima*	0.0062 ±0.00014	0.0066 ±0.00015	0.0058 ±0.00012	0.0061 ±0.00013	0.0049 ±0.00008	0.0059 ±0.00012
03	*Nostoc muscorum*	0.0100 ±0.00028	0.0095 ±0.00026	0.0093 ±0.00025	0.0089 ±0.00023	0.0090 ±0.00023	0.0093 ±0.00025
04	*Tolypothrix tenius*	0.0081 ±0.00020	0.0074 ±0.00018	0.0095 ±0.00027	0.0078 ±0.00019	0.0064 ±0.00015	0.0078 ±0.00020
05	Isolate 2	0.0033 ±0.00005	0.0044 ±0.00007	0.0032 ±0.00004	0.0029 ±0.00004	0.0023 ±0.00003	0.0032 ±0.00004
06	Isolate 3	0.0021 ±0.00002	0.0037 ±0.00005	0.0028 ±0.00003	0.0040 ±0.00006	0.0039 ±0.00006	0.0033 ±0.00005
07	Isolate 4	0.0052 ±0.00009	0.0057 ±0.00010	0.0060 ±0.00013	0.0059 ±0.00012	0.0045 ±0.00007	0.0055 ±0.00010

* S1, S2, S3, S4, & S5 are five different spots in the site.

Table-4.7 Determination of end point growth of nitrogen fixing *Heterocystous cyanobacteria*

S. No.	Cyanobacterial strains	Determination of Biomass, Chlorophyll and Total nitrogen		
		Biomass (mg/ml)	Chlorophyll (µg/ml)	Total Nitrogen (%)
01	*Anabaena variabilis*	0.38±0.0053	3.52±0.052	0.0075±0.00019
02	*Aulosira fertilissima*	0.48±0.0101	5.22±0.121	0.0059±0.00012
03	*Nostoc muscorum*	0.57±0.0131	6.37±0.162	0.0093±0.00025
04	*Tolypothrix tenius*	0.70±0.0182	2.45±0.030	0.0078±0.00020
05	Isolate 2	0.82±0.0230	7.12±0.195	0.0032±0.00004
06	Isolate 3	0.52±0.0114	4.18±0.075	0.0033±0.00005
07	Isolate 4	0.31±0.0037	4.37±0.090	0.0055±0.00010

Table-4.8 Wet Biomass (kg per m^2) in algal pond

S. No.	Incubation (Days)	Wet Biomass per unit area (kg per m2) in pond					
		S1	S2	S3	S4	S5	Mean
01	7	3.10±0.03	3.40±0.05	3.30±0.04	3.50±0.05	3.20±0.04	3.30±0.04
02	14	5.40±0.10	5.10±0.09	5.70±0.12	4.90±0.08	5.20±0.09	5.26±0.10
03	21	8.30±0.18	8.40±0.20	8.10±0.17	8.70±0.21	8.90±0.22	8.48±0.20
04	28	9.80±0.27	9.70±0.26	9.90±0.29	9.50±0.25	9.80±0.28	9.74±0.27

* S1, S2, S3, S4, & S5 are five different spots in the site.

Table-4.9 Dry Biomass (kg per m^2) in algal pond

S. No	Incubation (Days)	Dry Biomass per unit area (kg per m2) in pond					
		S1	S2	S3	S4	S5	Mean
01	7	0.51±0.006	0.56±0.008	0.55±0.007	0.58±0.009	0.53±0.006	0.55±0.007
02	14	0.93±0.018	0.85±0.014	0.95±0.020	0.81±0.013	0.86±0.015	0.88±0.016
03	21	1.38±0.030	1.40±0.034	1.35±0.028	1.45±0.035	1.48±0.037	1.41±0.033
04	28	1.63±0.046	1.61±0.043	1.65±0.048	1.58±0.041	1.63±0.047	1.62±0.045

* S1, S2, S3, S4, & S5 are five different spots in the site.

Table-4.10 Temperature, pH and water level in algal pond

S. No.	Incubation (Days)	Variation of pH in pond					
		S1	S2	S3	S4	S5	Mean
01	7	7.5±0.10	7.2±0.08	7.3±0.09	7.6±0.14	7.5±0.11	7.4±0.10
02	14	7.5±0.11	7.6±0.12	7.6±0.13	7.7±0.16	7.9±0.17	7.7±0.14
03	21	7.8±0.16	7.7±0.15	8.0±0.19	8.1±0.19	8.9±0.26	8.1±0.19
04	28	8.4±0.22	8.7±0.23	8.7±0.24	8.3±0.21	8.9±0.27	8.6±0.19
S. No.	Incubation (Days)	Variation of Temperature (^{0}C) in pond					
		S1	S2	S3	S4	S5	MEAN
01	7	25±0.73	23±0.51	22±0.29	21±0.25	22±0.37	22.6±0.43
02	14	23±0.44	25±0.73	22±0.31	24±0.65	23±0.58	23.4±0.54
03	21	23±0.48	23±0.55	24±0.62	22±0.35	24±0.67	23.2±0.54
04	28	23±0.48	21±0.23	22±0.33	23±0.55	22±0.40	22.2±0.40
S. No.	Incubation (Days)	Variation of Water level (inch) in pond					
		S1	S2	S3	S4	S5	MEAN
01	7	7.1±0.18	7.1±0.19	7.1±0.20	7.1±0.21	7.1±0.21	7.1±0.20
02	14	6.2±0.14	6.2±0.15	6.1±0.13	6.2±0.15	6.2±0.16	6.2±0.15
03	21	5.8±0.10	5.8±0.10	5.7±0.09	5.8±0.11	5.8±0.12	5.8±0.10
04	28	5.4±0.06	5.4±0.07	5.4±0.08	5.4±0.08	5.3±0.06	5.4±0.07

* S1, S2, S3, S4, & S5 are five different spots in the site.

Total nitrogen (%) estimation of heterocystous cyanobacterial strains at 7 days incubation period, shown maximum in *Anabaena variabilis* i.e. 0.0046±0.000115 mg/ml followed by Isolate 3, 0.0028±0.000067 mg/ml whereas minimum value recorded 0.0010±0.000012 mg/ml in Isolate 2, followed by *Nostoc muscorum* 0.0013±0.000020 mg/ml and Total nitrogen (%)estimation at 14 days incubation period, shown maximum in *Tolypothrix tenius* i.e. 0.0075±0.00019 mg/ml followed by *Anabaena variabilis*, 0.0073±0.00018 mg/ml whereas minimum value recorded 0.17±0.0016 mg/ml in *Nostoc muscorum*, followed by Isolate 4, 0.0018±0.00003 mg/ml whereas total nitrogen (%) estimation at 21 days incubation period, shown maximum in *Anabaena variabilis* i.e. 0.0113±0.00028 mg/ml followed by *Aulosira fertilissima*, 0.0075±0.00018 mg/ml whereas minimum value recorded

0.0012±0.00001 mg/ml in Isolate 3, followed by Isolate 4, 0.0029±0.00004 mg/ml. The results are tabulated in Table 4.2

Heterocyst frequency (%) of different cyanobacterial strains was analysed. Maximum heterocyst frequency (%) was recorded 30.10±0.873 % in S4 followed by 28.25±0.819 %, 27.40±0.767 % in S5 and S3. Minimum heterocyst frequency (%) was recorded 9.95±0.109 % in S4 followed by 10.25±0.113%, 10.40±0.125 % in S1, S2 separately, whereas spot wise heterocyst frequency (%) was recorded maximum in *Tolypothrix tenius* at S1 i.e. 22.10±0.619 % followed by 21.09±0.548 %, 27.40±0.0.767 %, 30.10±0.873 % & 28.25±0.819% at S2, S3, S4 & S5 and spot wise heterocyst frequency (%) was recorded minimum in Isolate 9 at S1 i.e. 11.27±0.147 % followed by 10.40±0.125 % at S2, 9.95±0.109% at S4, Isolate 8 was recorded in minimum in S3, i.e. 10.28±0.123%, Isolate 10 was recorded in minimum in S5, i.e. 14.10±0.240%. The results are tabulated in Table 4.3

Dry biomass estimation (mg/ml) of different heterocystous cyanobacterial strains was analysed. Maximum Dry biomass (mg/ml) was recorded 0.94±0.0273 mg/ml in Isolate 2 followed by 0.92±0.0276 mg/ml, 0.85±0.0247 mg/ml. Minimum Dry biomass (mg/ml) was recorded 0.15±0.0017 mg/ml in *Anabaena variabilis* followed by 0.29±0.0035 mg/ml, 0.30±0.0033 mg/ml in Isolate 4, whereas spot wise Dry biomass was recorded maximum in S1 i.e. 0.94±0.0273 mg/ml followed by 0.92±0.0276 mg/ml, 0.85±0.0247 mg/ml, 0.77±0.0216 mg/ml & 0.75±0.0203 mg/ml at S3, S2, S5 & S4 and spot wise Dry biomass mg/ml was recorded minimum in S2 i.e. 0.15±0.0017 mg/ml followed by 0.29±0.0035 mg/ml at S3, 0.30±0.0033 mg/ml at S1, 0.31±0.0040 mg/ml at S5 and 0.46±0.0083 mg/ml at S4. Biomass was expressed a dry biomass mg/ml basis that shows the growth of organism mostly an increasing trend. The results are tabulated in Table 4.4.

Chlorophyll estimation (µg/ml) of different heterocystous cyanobacterial strains after 21 days of incubation were analysed. Maximum Chlorophyll (µg/ml) was recorded in Isolate 2 i.e. 7.60±0.213 µg/ml followed by 7.24±0.203 µg/ml, 7.14±0.200 µg/ml. Minimum Chlorophyll (µg/ml) was recorded in *Tolypothrix tenius* i.e. 2.10±0.023 µg/ml followed by 2.12±0.023 µg/ml, 2.14±0.026 µg/ml, whereas spot wise Chlorophyll estimation was recorded maximum in S2 i.e. 7.60±0.213 µg/ml followed by 7.24±0.203 µg/ml, 7.14±0.200 µg/ml, 7.13±0.193 µg/ml & 6.48±0.168 µg/ml at S5, S3, S1 & S4 and spot wise Chlorophyll (µg/ml) was recorded minimum in S5<S1<S3<S4<S2 i.e. 2.10±0.023 µg/ml followed by 2.12±0.023 µg/ml, 2.14±0.026µg/ml, 2.80±0.036µg/ml and 3.10±0.040 µg/ml. The results are tabulated in Table 4.5

Total nitrogen content (%) of different heterocystous cyanobacterial strains after 21 days of inoculation were studied, the maximum total nitrogen found 0.0100±0.00028 % in *Nostoc muscorum* followed by 0.0095±0.00027 % in *Anabaena variabilis* & *Tolypothrix tenius* whereas minimum total nitrogen found in Isolate-3 i.e. 0.0021±0.00002% followed by 0.0023±0.00003%& 0.0029±0.00004% in Isolate-2, whereas spot wise total nitrogen was recorded maximum in S1, i.e. 0.0100±0.00028% followed by 0.0095±0.00026%, 0.0095±0.00027%, 0.0090±0.00023% & 0.0089± 0.00023% at S2>S3>S5>S4 and spot wise total nitrogen (%) was recorded minimum in S1<S5<S3<S4<S2 i.e. 0.0021±0.00002%, 0.0023± 0.00003%, 0.0028±0.00003%, 0.0029±0.00004% and 0.0037±0.00005%. The tabulated data are presented in Table No. 4.6.

After 21 days of inoculation end point growth of cyanobacterial culture was determined in reference of chlorophyll content biomass and total nitrogen among various cyanobacterial strains. It showed that the maximum biomass was recorded 0.82±0.0230 mg/ml in Isolate-2 and minimum biomass was recorded 0.31±0.0037 mg/ml in Isolate-4. The Chlorophyll content recorded maximum in Isolate-2 i.e. 7.12±0.195 µg/ml and chlorophyll content recorded minimum in *Tolypothrix tenius* i.e. 2.45±0.030 µg/ml whereas total nitrogen (%) was found maximum in *Nostoc muscorum* i.e. 0.0093±0.00025% and recorded minimum in Isolate-2 i.e. 0.0032±0.00004%. The results are tabulated in Table No. 4.7.

The wet biomass (kg/m^2) in different algal ponds was analysed. The average wet biomass recorded minimum in 7 days incubation period i.e. 3.30±0.04 kg/m^2, followed by 5.26±0.10 kg/m^2 in 14 days incubation period, 8.48±0.20 kg/m^2 in 21 days incubation period and recorded maximum in 28 days incubation periods i.e. 9.74±0.27 kg/m^2. This indicates that wet biomass increased with in time of incubation period. The spot wise maximum wet biomass value recorded 3.50±0.05 kg/m^2 in S4, and recorded minimum 3.10±0.03 kg/m^2 in S1 in 7 days incubation period, and 14 days incubation period shows maximum wet biomass in S1 i.e. 5.40±0.10 kg/m^2 and recorded minimum in S4 i.e. 4.90±0.08 kg/m^2, whereas 21 days incubation period shows maximum wet biomass in S5 i.e. 8.90±0.22 kg/m^2 and recorded minimum in S3 i.e. 8.10±0.17 kg/m^2 and 28 days incubation period shows maximum wet biomass in S3 i.e. 9.90±0.29 kg/m^2 and recorded minimum in S4 i.e. 9.50±0.25 kg/m^2. The results are tabulated in Table 4.8.

The dry biomass (kg/m^2) in different algal ponds was analysed. The average dry biomass recorded minimum in 7 days incubation period i.e. 0.55±0.007 kg/m^2, followed by 0.88±0.016 kg/m^2 in 14 days incubation period, 1.41±0.033 kg/m^2 in 21 days incubation period and recorded maximum in 28 days incubation periods i.e. 1.62±0.045 kg/m^2. This indicates that dry biomass increased with in time of incubation period. The spot wise

maximum dry biomass value recorded 0.58±0.009 kg/m^2 in S4, and recorded minimum 0.51±0.006 kg/m^2 in S1 in 7 days incubation period, and 14 days incubation period shows maximum dry biomass in S3 i.e. 0.95±0.020 kg/m^2 and recorded minimum in S4 i.e. 0.81±0.013 kg/m^2, whereas 21 days incubation period shows maximum dry biomass in S5 i.e. 1.48±0.037 kg/m^2 and recorded minimum in S3 i.e. 1.35±0.0028 kg/m^2 and 28 days incubation period shows maximum dry biomass in S3 i.e. 1.65±0.048 kg/m^2 and recorded minimum in S4 i.e. 1.58±0.041 kg/m^2. The results are tabulated in Table 4.9.

The variation of pH in different pond was analysed. The average pH recorded minimum in 7 days incubation period i.e. 7.4±0.10, followed by 7.7±0.14 in 14 days incubation period, 8.1±0.19 in 21 days incubation period and recorded maximum in 28 days incubation periods i.e. 8.6±0.19. This indicates that pH increased with in time of incubation period. The spot wise maximum pH value recorded 7.6±0.14 in S4, and recorded minimum 7.2±0.08 in S2 in 7 days incubation period, and 14 days incubation period shows maximum pH in S5 i.e. 7.9±0.17 and recorded minimum in S1 i.e. 7.5±0.11, whereas 21 days incubation period shows maximum pH in S5 i.e. 8.9±0.26 and recorded minimum in S2 i.e. 7.7±0.15 and 28 days incubation period shows maximum pH in S5 i.e. 8.9±0.27 and recorded minimum in S4 i.e. 8.3±0.21. The results are tabulated in Table 4.10.

The variation of temperature in different pond was studied. The average temperature recorded minimum in 28 days incubation period i.e. 22.2±0.40 ^{0}C, followed by 22.6±0.37 ^{0}C in 7 days incubation period, 23.2±0.54 ^{0}C in 21 days incubation period and average temperature recorded maximum in 14 days incubation periods i.e. 23.4±0.54 ^{0}C. This indicates that average temperature increased with in time of incubation period. The spot wise maximum temperature value recorded 25±0.73 ^{0}C in S1, and recorded minimum 21±0.25 ^{0}C in S4 in 7 days incubation period, and 14 days incubation period shows maximum temperature in S2 i.e. 25±0.73 ^{0}C and recorded minimum in S3 i.e. 22±0.31 ^{0}C, whereas 21 days incubation period shows maximum temperature in S3 & S5 i.e. 24±0.62 ^{0}C, 24±0.67 ^{0}C and recorded minimum in S4 i.e. 22±0.35 ^{0}C and 28 days incubation period shows maximum temperature in S1 & S4 i.e. 23±0.48 ^{0}C, 23±0.55 ^{0}C and recorded minimum in S2 i.e. 21±0.23 ^{0}C. The results are tabulated in Table 4.10.

The variation of water level (inch) in different pond was studied. The average water level recorded minimum in 28 days incubation period i.e. 5.4±0.07 inch, followed by 5.8±0.10 inch in 21 days incubation period, 6.2±0.15 inch in 14 days incubation period and average water level recorded maximum in 7 days incubation periods i.e. 7.1±0.20 inch. Level of water shows decreasing trend when time of incubation period is increased. The spot wise maximum water level recorded 7.1±0.21 inch in S4 & S5, and recorded minimum 7.1±0.18 inch in S1 in 7 days incubation period, and 14 days incubation period shows maximum water level in S5 i.e. 6.2±0.16 inch and recorded minimum in S1 i.e. 6.1±0.13 inch, whereas 21 days incubation period shows maximum water level in S5 i.e. 5.8±0.12 inch, and followed by 5.8±0.10 inch in S1 & S2 and recorded minimum in S3 i.e. 5.7±0.09 inch and 28 days incubation period shows maximum water level in S3 & S4 i.e. 5.4±0.08 inch, and recorded minimum in S5 i.e. 5.3±0.06 inch. The results are tabulated in Table 4.10.

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